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ON

PUBLIC HEALTH AND MEDICAL SUBJECTS.

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MINISTRY OF HEALTH.

LONDON:
PUBLISHED BY HIS MAJESTY'S STATIONERY OFFICE.
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Presented by

Sir George Newman

July 1923.

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PREFATORY NOTE BY THE CHIEF MEDICAL OFFICER.

To The Right Hon. Neville Chamberlain, M.P.,
Minister of Health.

Sir,

1. I beg to submit two reports from the Ministry's Pathological Laboratory which form additional contributions to the study of epidemiological problems by bacteriological methods.

2. Dr. Griffith has discovered a new and simple way to distinguish colonies of virulent and non-virulent pneumococci, and has shown that strains derived from such different colonies differ not only in virulence but in their antigenic characters. This observation is of great importance in the choice of strains for the preparation of protective and therapeutic sera, since sera prepared with strains from the non-virulent colony have almost no protective action against infections with the virulent strain.

Further, Dr. Griffith has found that exposure of a virulent strain to the action of a specific immune serum invariably leads to the appearance of non-virulent colonies, and, if the exposure is prolonged, to the transformation of the virulent pneumococci into an entirely non-virulent strain. This fact suggests that here we have one, at least, of the ways in which immune serum exerts its protective action in the animal body.

3. These observations are of wider interest, however, as a contribution to the study of bacterial variation. In recent years much attention has been given by bacteriologists to elucidation of causes which determine variations in the virulence of bacteria. The disconcerting fact has been brought to light that a pure culture, bred from a single colony or from a single bacterium, is not necessarily a collection of homogeneous individuals; it is often an aggregation of units which, though all true to species, differ from each other in certain biological respects amongst which is the property termed "virulence." It has been shown that no simple explanation of this phenomenon is possible owing to the many influences concerned.

4. Dr. Eastwood has endeavoured to extricate some general principle from the confusing mass of data which have accumulated around the subject of bacterial variation. He shows that these observations of Dr. Griffith can be brought into line with other

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recent work on bacterial variants, including the much discussed "Twort d'Herelle phenomenon" of transmissible bacterial autolysis. He points out that all these different manifestations of bacterial changes have one factor in common; the changes only occur in bacteria which are actually growing. He therefore concludes that they must be due to some interference with vital processes at the nascent stage of bacterial growth. On this assumption he proceeds to formulate a working hypothesis which may help to elucidate general principles. The constituents of living bacterial protoplasm have two functions (1) Catalytic *i.e.*, the preparation of food material with the aid of their appropriate enzymes; (2) Synthetic, *i.e.*, the building up of new protoplasm. Any disturbance of the delicately adjusted balance between these two activities, whether by change of environment, pabulum or otherwise, must tend to the production of variants. In his report Dr. Eastwood furnishes a lucid account of the known facts concerning bacterial variants and discusses them in relation to the interesting hypothesis which he has formulated.

I have the honour to be,

Sir,

Your obedient servant,

GEORGE NEWMAN.

Whitehall,
June, 1923.

BACTERIOLOGICAL STUDIES.

indistinguishable in appearance, there seems to me little doubt that both he and Arkwright are dealing with essentially the same phenomenon and that the differences in some of the results of the two workers merely represent different stages in the tendency of a culture to separate into individuals with distinctive characters.

The varieties of pneumococcus colonies which I am about to describe show differences in morphology and in some of their immunological reactions, which are similar to those found by Arkwright in other bacterial species. They were discovered in the course of my investigation of the mode of action of anti-pneumococcus serum. On plating cultures of pneumococci grown on homologous antisera, it was found that a number of colonies which differed from the normal in appearance were attenuated in virulence for mice.*

Attenuation was definitely associated with the production of rough colonies, and these could be clearly distinguished from those of smooth appearance which retained their virulence.

Culture Media.

Plate cultures.—Nutrient agar prepared with trypsinised meat was used, with the addition of 5 per cent. chloroformed whipped horse blood and 5 per cent. filtered horse serum. On this medium isolated colonies of pneumococci grow up to 2 mm. in diameter, and autolysis is often very slight.

The horse blood should be collected from a vessel directly into a sterile bottle, since chloroform fails to get rid of gross contamination. The blood cells, which rapidly deposit, are syphoned off into medicine bottles, and about 2 per cent. chloroform is added : it is useful to have some glass beads in the bottle to break up the cells, which form a thick mass under the action of the chloroform. The horse serum is filtered through a Berkefeld candle.

Blood broth.—Colonies from plates are grown in small tubes, 3 inches by $\frac{1}{2}$ inch, containing about 1 c.c. of equal parts of whipped rabbit blood and trypsinised meat broth. Growth occurs rapidly in the small bulk of medium, and can be recognised by the medium becoming dark purplish in colour. The blood cells keep almost intact in the ice chest for several weeks, and are useful in showing the presence or absence of hæmolysis due to bacterial growth. After inoculation and incubation the cells fall to the bottom of the tube, and a loopful of the deposit always produces a good subculture of pneumococci in a tube containing about 6 c.c. of ordinary broth.

Immune Serum.—The sera are prepared in rabbits from virulent cultures grown for 4–6 hours in glucose broth. The broth cultures are heated to 60° C. for an hour and centrifuged;

* Laura Stryker (*Journ. Exp. Med.*, xxiv, p. 49, 1916) had already observed that repeated subculture of Types I. and II. pneumococci in homologous serum caused marked reduction in virulence.

the supernatant broth is syphoned off, leaving about 15 c.c. out of 100 c.c., in which the deposit is re-suspended. Rabbits receive 5 c.c. intravenously for each injection; the injections are given in series on 2 or 3 consecutive days and at weekly intervals, and after about 2–3 months a usable serum is often produced.

For use as a culture medium the serum is distributed in small tubes in quantities of 0·5 c.c., either neat or diluted as desired. A few drops of a thick suspension of red cells (rabbit) are added to favour growth and serve as an indicator.

Cultivation of Pneumococci in Immune Serum.

Several serological types of virulent pneumococci have been grown in immune serum, though the majority of the observations recorded have been made upon stock strains of Types I. and II., which were obtained from the Rockfeller Institute. The action of the homologous serum upon a pneumococcus culture has been controlled by simultaneous experiments with heterologous sera. After each incubation at 37° C. overnight, the serum cultures were sown on plates of blood agar so as to obtain discrete colonies. With very few exceptions, which will be referred to later, no alteration was observed in the characters of the colonies obtained after growth in a heterologous serum, and smear preparations showed that no agglutination had taken place, the pneumococci being distributed uniformly throughout the medium. The possibility that heterologous sera produced some biological modification not associated with morphological change has not been investigated. In the homologous serum growth took place in the form of clumps and long chains or sometimes as translucent gelatinous balls which deposited at the bottom of the tube. When a first culture in serum was shaken and plated, in addition to the normal S type of colony, a variable number of colonies appeared which differed from them in size, appearance and consistency. These were the R form. A second serum culture made from the first yielded, when plated, a larger number of variants from the normal type. After a third passage in serum the normal S form was replaced by the R. Generally the pneumococci at this stage had ceased to clump, and were tending to grow uniformly throughout the medium. When a loopful of the first culture in serum, which, as stated above, usually consists, after a night's incubation of a mixture of R and S forms, is sown into plain blood broth, serial cultures up to the 13th generation have remained a mixture, the normal type in this instance showing a tendency to decrease in numbers relative to the R type.

A single colony culture of either type, made at any stage of the treatment with serum, as a rule, reproduces only the same type when sown into plain blood broth. This rule is not without exceptions, and occasionally, when the single colony culture of the variant has been made after a single serum passage, a reversion of some of the colonies to the normal smooth type has been observed.

The question of the stability of the R and S characters will be considered later.

In order to produce the variant from a normal strain through the influence of specific serum, it is necessary that growth should occur. No alteration has been observed on plating a culture that has remained in contact with the specific serum at ice-chest temperature, or has been shaken with the serum at room temperature. The variant, therefore, is a new generation in the serum and not one of the original pneumococci altered by contact with the serum. This point is of importance when one compares the action of the serum upon the pneumococci in the animal body and in the test tube.

Concentrated serum is not essential for the production of R forms. A dilution of 1 in 256 has caused a partial change of a smooth culture into a rough even with a single treatment. In addition, the blood of a mouse, which has received a dose of 0·2 c.c. of serum, has been collected about 18 hours later, and used as a culture medium. The pneumococci sown into it grew as in a dilute agglutinating serum, and from one such culture, after being incubated a night and then plated, several rough colonies were produced.

Heterologous sera, as already stated, have generally no power to produce rough colonies from a smooth culture and numerous experiments have given negative results, even after several passages. I have, however, found one exceptional instance. A Type I. strain grown in a Type II. serum produced rough colonies when plated; these remained rough after many generations in blood broth. Until then it had seemed that the production of rough colonies in a given serum might serve as a test of the serological type. For example, the atypical II. A and II. B strains, which are believed by the American observers to be related to Type II., were grown in undiluted Type II. serum. Both strains grew uniformly and not in chains, thus showing the absence of agglutinins for them, and after five consecutive passages in Type II. serum the colonies remained of the normal smooth type. I have recorded elsewhere* evidence that the so-called atypical Type II. strains are serologically distinct from Type II.

Description of the R and S Forms of Pneumococcus Colonies.

All the serological varieties of pneumococci with the exception of Type III. yield on the same medium colonies which are indistinguishable. There is, however, only a difference in degree between the Type III. colonies and the others, and sometimes the colonies of Type II. and the atypical strains of Group IV. are equally large and watery. The following description of the normal type of colony applies only to strains which have been

* *Reports on Pub. Health and Med. Subjects, Ministry of Health*, No. 13, 1922.

kept in a virulent condition by repeated passage through animals and maintenance in culture on favourable media. The medium upon which the special differential features of the colonies are best shown is the chocolate-coloured serum agar with chloroformed blood cells added. On ordinary agar, or on agar smeared with fresh blood, the different varieties of colonies are much less readily distinguished.

When the blood from a mouse which has died from pneumococcus septicæmia, or a young blood-broth culture, is plated on the above medium and incubated overnight, all the discrete colonies are of the same type. They are circular, dome-shaped, with a smooth shiny surface and an almost watery consistency; when autolytic changes take place they become flattened, and finally shaped like a button or draught. The characteristic feature of the variant or rough type of colony is the surface, which is dull or finely granular; the colony is more coherent than the smooth type, and may either break into pieces on being touched or stick as a whole to the point of the needle. The difference in appearance between the two varieties of colonies on the same plate is often very striking, but it is essential that the colonies should be well spaced. At first the rough variant is generally smaller than the smooth colony: it is equally bile-soluble.

Such are the two types of colonies which can be grown from a normal virulent strain cultivated in homologous immune serum. There is a close resemblance between the changes described above and those described by Arkwright as occurring in old cultures of various bacilli of intestinal origin. In the case of pneumococci, as with these, rough colonies can be produced also by growing cultures on agar media or in liquid media containing glucose. Arkwright, as mentioned earlier, designated his types of colonies S (smooth) and R (rough) respectively, and this seems quite appropriate for the pneumococcal colonies, the S being the normal and the R the variant. There is, however, a slight distinction, which is probably one of degree, namely, that the rough pneumococcal cultures grow uniformly in broth and form stable suspensions, whereas Arkwright's R colonies form clumps in any medium containing 0·85 per cent. salt solution. The rough cultures in broth are, however, a little more granular than the smooth; they clear more completely by centrifuging, and the deposit packs into a mass, which is not so readily emulsified as the deposit from the smooth culture.

Stability of R and S Characters.

The smooth characters of the normal colonies remain unaltered so long as the strains are kept virulent by passage through animals and growth in blood broth. They persist even after prolonged culture on solid media. For example, a number of cultures were allowed to remain in sealed tubes of ordinary agar in the incubator at a temperature of 37° C. At the end of three months all were

still viable; on plating, some yielded apparently all smooth colonies, and, where rough colonies were detected, they were few in number. A rough colony culture, which has been produced from a virulent strain by a single passage in immune serum, may retain its rough characters for many generations in plain blood broth and, as already mentioned, may subsequently revert to the smooth type. This has occurred particularly with Type II., and the reversion in cultural characters has been associated with the recovery of virulence. Certain of the R colony strains, which have been subcultivated for many generations in plain blood broth and have been plated at intervals, have become in appearance almost indistinguishable from the S strain, but they have remained attenuated.

The change in the biological characters is of greater importance than the morphological differences between the colonies. On the other hand the characteristics of the R type obtained by three passages through immune serum have been maintained for more than 13 subsequent generations in plain blood broth. The activity of the immune serum, *i.e.*, the height of its titre, appears to have an influence upon the permanence of the change.

Virulence.

So far as my observations go at present the typical S colony derived from a virulent culture is always pathogenic for mice though one cannot affirm that all S colonies are equally virulent. There is some evidence that a partial degree of attenuation is associated with a corresponding tendency to the rough state, and such partially rough colonies yield typically smooth colonies after causing septicæmia in a mouse. The typical R colony shows, on the other hand, a high degree of attenuation. When a virulent pneumococcus is grown overnight in immune serum, a plate made from the serum culture yields a mixture of R and S colonies. If a colony of each type is selected and subcultivated for several generations in plain blood broth, the S colony culture is found to be as virulent as the original culture, *e.g.*, killing mice in doses of 0·000,000,1 c.c. and 0·000,000,01 c.c. of broth culture, while the R culture has no effect in doses of 0·1 c.c. and 0·2 c.c. (the latter being the greatest amount inoculated).

Retested on mice after 18 generations in blood broth, the above cultures gave similar results: the R culture failed to kill mice in doses ranging up to 0·25 c.c. of blood broth culture, while the S culture had remained apparently unaltered in virulence. If a mouse dies of typical septicæmia after an inoculation of an apparently typical R culture, the colonies grown from the blood are invariably of the S type, that is, the culture has reverted. Occasionally, however, a mouse may die after a large dose of R culture, and the only organisms recoverable from the blood are a few colonies of the R type. In such a case the peritoneal washings do not give the characteristic agglutino-precipitin reaction with the type serum prepared from the normal strain, and the blood of

the mouse produces no effect when inoculated into a second mouse. The following example shows how repeated passage through immune serum fixes the attenuated character. A colony of a Type III. strain, virulent for mice in a dose of 0·000,000,1 c.c. of broth culture, was cultivated for one night in Type III. serum; a second serum culture was sown from the first. Both serum cultures were plated when 24 hours old, and an R colony was subcultivated from each in plain blood broth. (The R colonies of a Type III. strain are readily distinguished from the large watery S colonies.) Each culture was tested on mice. The R culture from the first serum killed mice, and was found on being recovered from the blood to have reverted to the S type. The colony culture from the second serum was completely attenuated, and failed to kill mice in a dose of 0·2 c.c.

Antigenic Characters of R and S Cultures.

My observations have not been numerous but they show that the two types of colonies have different antigenic qualities. Sera have been prepared in rabbits with the R and S cultures of both Type I. and Type II. The results with Type I. sera, which I am about to describe, were also obtained with the first samples of Type II. sera. Later samples of the Type II. sera showed less difference between the R and S sera, and on investigation it was found that the R culture used for immunisation had partially reverted to the S type. The Type I. sera were prepared with strains which retained their special characters unaltered during the period of immunisation. Three rabbits were immunised with R culture, and one with S culture: the cultures were heated to 60° C., and were prepared freshly before each injection. All the rabbits received identical treatment, 12 injections being given intravenously during a period of five weeks. Samples of blood were withdrawn eight days after the last inoculation.

Agglutination.—The serum from the rabbit injected with the S culture agglutinated both S and R cultures up to a dilution of 1 in 160. The product of the reaction between the serum and the S culture consisted of firm masses, which could not be broken up by shaking. The R culture agglutinated with the formation of loose clumps which were readily broken up on shaking to form a finely granular suspension.

Of the three rabbits injected with R culture one only yielded a good agglutinating serum. This agglutinated the homologous R strain up to 1 in 640, and the clumps formed were of the same loose character as those described above in the case of the reaction between the R culture and the S serum. The S culture was barely agglutinated in 1 in 10 with the R serum.

Precipitin.—The sera were tested upon broth culture which had been freed from cocci by centrifuging and filtration through a Berkefeld candle. When the S serum was mixed with the filtrate of the S culture, a precipitate was formed which aggre-

gated into a firm mass; with the filtrate of the R culture there was no visible reaction.

The serum prepared with the R culture gave a precipitate neither with the R nor with the S filtrate.

Absorption of Agglutinin.—The serum of the rabbit immunised with S culture lost after treatment with S culture all its agglutinins, *i.e.*, both for R and S suspensions. Treatment with the R culture removed the agglutinin for the R and left unaltered the agglutinating capacity of the serum for the homologous S strain.

The serum of the rabbit prepared with the R strain required two successive treatments with the R culture before it lost the whole of its homologous agglutinin. Similar treatment with the S culture did not exhaust the homologous agglutinin from the R serum, but reduced the titre for the R strain from 1 in 640 to 1 in 160.

Protection of Mice with R and S Sera.—The S serum, injected intraperitoneally into mice in doses of 0·2 c.c., protected them against a subsequent inoculation of doses of a Type I. strain ranging up to 0·1 c.c. of broth culture, the virulence of which was such that 0·000,000,01 c.c. of broth culture killed an unprotected mouse within two days. Against the same strain of Type I. all the R sera failed to show any protective power. The inoculation of the three rabbits with R culture was continued in order to ascertain whether more prolonged treatment would induce the development of antibodies against the virulent strain. The three rabbits were retested after they had been immunised for a total period of four months, during which each rabbit received 26 intravenous injections. Their sera still failed to show the presence of protective substances, when tested against a Type I. culture which killed mice in a dose of 0·000,000,01 c.c. Two other rabbits, which had been injected with the S culture for five weeks and were tested at the same time, yielded sera which protected mice against doses of 0·1 c.c. of the above culture.

Influence on the Type of Colony by Growth in R and S Sera.

A virulent Type I. culture was grown in R serum and in S serum. There was no alteration in the character of the colonies obtained on plates after three successive passages through the R serum. On the other hand the culture grown in the S serum, when plated after incubation overnight, yielded a mixture of R and S colonies. One of each variety was subcultured in plain blood broth, and was tested for virulence on mice. The S strain killed mice in a dose of 0·000,000,1 c.c. of broth culture, while 0·2 c.c. of the R strain proved innocuous.

Agglutinability of R and S Strains.

The clumps formed after the interaction between a virulent pneumococcus culture and homologous immune serum are very characteristic. On the addition of a 1 in 10 dilution of the serum

to pneumococcus broth culture there is an immediate turbidity, and visible clumps quickly form, especially on gentle shaking, which aggregate into a compact mass. An almost identical result, but a less bulky precipitate, is obtained with a broth culture from which the cocci have been removed by filtration. Thus, this type of reaction, which is very specific for each serological type of pneumococcus, depends on the formation of a precipitate between the serum and a soluble substance in the broth. If, however, the centrifuged cocci from a broth culture are resuspended in salt solution, they form a compact mass with immune serum similar to the above, although the soluble substance has been removed with the broth. This appears to indicate that either the external portion of the pneumococcus contains some of the soluble substance in process of secretion, or both are of similar constitution.

The attenuated R strain agglutinates with the same serum in a different manner. If broth culture is used, agglutination is slow, and fine granules form which settle to the bottom of the tube. A more copious deposit is formed when a thicker salt solution suspension of cocci is used. In both cases, however, the clumps do not aggregate into a compact mass, but can be readily shaken up into a finely granular suspension. The difference in character between the reactions of the R and S strains seems capable of a simple explanation. Since no precipitation or turbidity occurs when the serum is added to a filtered broth culture of the R strain, it is obvious that the R culture does not form any soluble substance, and hence there is nothing to bind the agglutinated cocci into a firm mass.

The R culture suspensions are rather less stable than the smooth and, in addition, they are less specific in their agglutinability, often showing some reaction with heterologous sera; they continue, however, to react with the type serum to a higher titre than with any other pneumococcal serum.

Mode of Action of Protective Antipneumococcus Serum in the Animal Body.

The attenuation of virulent pneumococci by growth in homologous immune serum *in vitro* is a phenomenon which is probably related to the protective action of the serum in the animal body and may help to explain it, though the two processes are obviously not identical. In the test-tube the action of the serum is shown in the altered virulence of the new generation of pneumococci, hough the change is never complete in the first passage through serum, the culture remaining a mixture of virulent and non-virulent elements. Though pneumococci have been recovered from an immunised animal at several stages before their final disappearance it has not been possible actually to demonstrate any change into rough colonies. In an immunised animal the inoculated pneumococci have generally disappeared from the peritoneal cavity within 24 hours; they remain alive

longer when inoculated into the subcutaneous tissues, where they give rise to a localised abscess.

The following is the record of an unsuccessful attempt to demonstrate the production of rough forms of pneumococci in an immunised mouse. A virulent Type I. culture in broth was mixed with an equal quantity of a Type I. serum and incubated for half an hour, after which the mixture was inoculated intraperitoneally into two mice. A small amount of peritoneal fluid was withdrawn from each mouse one and two hours later, part of which was sown on plates. Only smooth colonies of virulent type grew, but mice inoculated with the rest of the fluids remained well. The two original mice were killed 20 hours after inoculation and the peritoneal cavities were washed out with salt solution. The centrifuged deposits from the washings were sown on blood agar plates; no growth of pneumococci was obtained.

If there is an intermediate stage in the mechanism of protection during which rough attenuated pneumococci, that is, cocci which have not developed the part of their external structure associated with the secretion of soluble substance, are produced in a passively immune mouse, it is clear that they would readily be destroyed, since even large doses of these R cocci inoculated into an unprotected mouse quickly disappear.

On the other hand, it may not be necessary to postulate such an intermediate stage. The immune serum may protect the mouse by depriving the pneumococcus of its virulent potentialities, possibly by precipitating the external covering associated with capsule formation and the secretion of soluble substances. The pneumococci so affected would become the prey of the phagocytes. They would, if removed from the animal before destruction, be capable of multiplying, and as the exposure to the immune serum had been brief, they would produce normal virulent descendants. But if within the animal body some sensitised pneumococci escaped the attacks of the leucocytes and divided, the descendants would remain under the influence of the serum, in which case the property of the serum to produce progressive attenuation might be brought into action.

Discussion of Results.

1. The first point of interest which emerges from this study of S and R forms of pneumococci is the fact that a definite morphological criterion has been found to distinguish between virulent and attenuated strains. Further evidence of attenuation is furnished by serological reactions, in particular by the loose character of the clumps in the agglutination test, and by the absence of specific soluble precipitable substance. Given these data, it will be possible to decide whether a culture is in the condition suitable for the production of disease.

2. An important practical corollary will be that, for the production of good immune sera, strains should be used which consist entirely of S forms. Immunisation of rabbits with the

R form has up to the present stage of the investigation failed to stimulate the production of protective substances against a virulent strain, whereas in the same time the injection of the S form produced a strongly protective serum.

3. As regards the influences which cause attenuation of virulence, I have found that this result can be produced invariably by growth in specific immune sera. I have also noted that R forms occasionally appear after prolonged residence on ordinary solid media. It is possible that similar changes may arise under natural conditions, as, for example, during convalescence from pneumonia. The study of the various influences which cause exaltation or attenuation of virulence may be facilitated by observing the occurrence of S and R colonies under different conditions.

4. This alteration from an S to an R type appears to be a natural tendency with many species of bacteria. It can, I think, be attributed to degenerative changes and is associated with the loss of certain antigenic qualities. For example, while studying the serological types of meningococci,* I subcultured separately 40 colonies of identical appearance and tested them against an agglutinating serum prepared with the whole strain. With one exception, all the daughter colonies absorbed the homologous agglutinin. The exceptional colony, although it agglutinated as well as any of the others, failed to lower the titre of the serum for the parent strain. This last result I considered to be evidence of a diminution of antigenic complexity. Now the relationship of the R pneumococcus to the S is in some respects similar to that of the variant meningococcus to the whole culture. A serum prepared with the stock culture or with the S form of pneumococcus agglutinates both R and S to the same titre. The S form removes the whole of the agglutinin both for R and S, but absorption with the R form has no effect on the titre of the serum for S. The S and R possess related antigens, as is shown by the agglutination test, but the R lacks an antigen which is contained in the S. Before it can be said that the R is simply an S form from which part of the latter's antigen has been removed, it must be shown that the R type of antigen is contained within the S form. A serum, prepared with R. and agglutinating that form up to 1 in 640, barely agglutinates the S form; if present in the S form the R antigen therefore is not available, or its union with the R antibody does not result in agglutination of S. Still, repeated absorption of R serum with S reduces somewhat the agglutinin for R, thus indicating that there are receptors of the R type present in the S.

5. What are the distinguishing features of the part of the antigen of the pneumococcus which is concerned in virulence? As one would naturally expect, in view of the intimate association of a capsule with virulence, it appears to be the outer portion

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* *Journal of Hygiene,* XVII., p. 166, 1918.

of the pneumococcus which is modified in the change from S to R. After growth in immune serum the virulent pneumococcus colony loses its smooth shiny surface and almost watery consistency and becomes dull and finely granular. That is to say, the individual pneumococci tend to adhere and are not separated by some substance which, probably on account of its being soluble in salt solution, permits the formation of a very fine stable emulsion. In addition, the attenuated R form no longer secretes in young broth cultures a soluble substance which reacts with an antiserum from a virulent strain. There is some evidence, therefore, that the R form, which is undoubtedly derived from the S, is differentiated from the latter by the loss of that part of its antigen which is concerned with virulence, capsule formation and secretion of specific soluble substance. The R form may be considered to be a stage in the degeneration of a pneumococcus from a virulent complex type to an attenuated form with a simpler antigenic structure.

6. The next observation of interest is that this loss is not necessarily permanent. Though in certain cases, as in old cultures and after repeated serum treatment, the R forms are apparently stable, it has been found, under other circumstances, that this alteration in an important biological function can apparently be reversed, not only by animal passage but also by continued subculture in blood broth. After many generations the R pneumococci assume again their smooth colony formation and at the same time their virulence is restored. Can it be said, in explanation of such results as these, that the original R colony contained a few virulent pneumococci which escaped notice in the earlier subcultures? This hypothesis, in view of the negative results of the periodical tests for virulence, cannot be regarded as probable. Yet it is difficult to understand how an attenuated organism can recover its virulence simply by repeated subculture without animal passage. A somewhat similar event has been described by Bail* in his work on the formation of capsules by B. anthracis. If a culture of anthrax bacilli is exposed to a temperature of 42° C. for a period which falls short of depriving it completely of the power to produce capsules, certain strains are formed which produce a mixture of colonies. Some of these colonies produce cultures all of which invariably fail to form capsules, while others produce in addition a few typical capsule-forming bacilli. This result, he thinks, depends upon a deficient inheritance of the capsule-forming substance, so that an individual bacillus is able to endow only one of its descendants with a sufficient amount to produce a typical capsule-forming strain.

7. Does this investigation throw any new light upon the protective action of pneumococcus immune serum? According to Neufeld's bacteriotropic theory the pneumococci after inoculation into an immunised animal become sensitised and are then des-

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* *Centralbl. f. Bakt.*, Orig., LXXIX., p. 425, 1917.

troyed by phagocytosis. Experiments *in vitro* provide supporting evidence, since virulent pneumococci are not ingested by leucocytes except in the presence of immune serum, and the leucocytes are thought to play an essential part in the mechanism and not to act merely as scavengers.

Perhaps the above observations on the attenuating action of immune serum may serve to amplify Neufeld's theory, the suggestion being that the action of immune serum is not entirely bacteriotropic. A certain number of pneumococci may not be directly subjected to phagocytosis in the presence of immune serum. These may give rise to new generations in the animal body, but such bacteria would, under the influence of the immune serum, be subjected at the onset of growth to rapidly progressive attenuation and would soon be destroyed.

Summary.

1. Virulent pneumococci become attenuated by growth in homologous immune serum.

2. The loss of virulence is associated with an alteration of the immunological characters.

The attenuated pneumococcus does not stimulate the production of protective substances in the blood of inoculated rabbits.

3. There is a morphological distinction between the colonies of the virulent and the attenuated pneumococci. The virulent colonies are smooth and the attenuated rough in character, the distinction being similar to that between the R and S varieties of B. dysenteriæ, &c. described by Arkwright.

4. The S pneumococcus produces in young broth cultures a specific soluble substance; the R pneumococcus does not.

5. The S form agglutinates with specific serum, producing a firm gelatinous precipitate; the R form produces loose clumps which are readily shaken up.

6. An R strain may revert in all respects to the S type, or may remain unchanged after many generations in subculture in plain blood broth. On the other hand, the morphological distinction between the R and the S forms may tend to disappear, while the immunological differences persist.

7. The attenuation of pneumococci by immune serum in the test-tube indicates that the mode of action of the serum in a protection experiment is a direct one upon the pneumococcus.

A double action may be suggested:—

(*a*) The serum may disorganise the biological functions of the pneumococcus by precipitating the capsule, thus inhibiting the secretion of anti-leucocytic substances and rendering it temporarily harmless.

(*b*) When pneumococci divide in the animal body in the presence of immune serum, it is suggested that the influence of the serum may cause progressive attenuation of subsequent generations.

November, 1922.

II.—BACTERIAL VARIATION AND TRANSMISSIBLE AUTOLYSIS; THE RELATION OF BACTERIAL ENZYMES TO BACTERIAL STRUCTURE.

By Arthur Eastwood, M.D.

Introduction.

Careful investigation of cultures, grown on ordinary or on selective media, has shown that variations are of common occurrence in most bacterial species. The variants, whilst retaining the general characters of their species, exhibit differences in one or more of the following attributes* :—appearance of the colonies, morphology of individual bacteria, motility, fermentative capacities, capacity for growth, antigenic properties, and virulence.

Of the principles which determine these changes very little is known, but certain laboratory data seem to be clearly established. In the first place, variants can be produced in cultures derived from a single cell or from single colonies, a procedure which has been repeated so often that there can be no doubt as to its accuracy; thus one has no difficulty in answering the question, Have you really produced a variant, or was the "variant" present in small numbers, which were unrecognised, in the material with which you started? The answer is that variants undoubtedly arise *de novo* and that this fact has been proved by properly controlled experiments, which show that variants are definitely attributable to some change associated with bacterial growth. In the next place, though variants observed in the laboratory are not all of equal importance and though some of them may be merely artefacts, in the sense that they are attributable to causes not likely to be operative in nature, the main fact remains that many of these variants are of great interest, because they throw light on the biological properties of bacteria and are thus related to the problems of infection, resistance and immunity.

To take the most obvious instance. A strain is split up into two types, the "normal" and a variant; though each grows equally well in subculture, when tested on animals it may be found that the former has retained its virulence whilst the latter has lost it. This observation is clearly of interest in discussing the factors on which virulence depends. Then one may go a step further. Under the operation of certain influences *in vitro*, a culture may be separated into two types, the "sensitive" and the "resistant," which do not grow equally well, even in the test-tube. The former is much less viable and the sensitive

* I am assuming, provisionally and for the sake of simplicity, that the attributes of the normal bacterium are clearly and accurately defined. This, however, is not always the case; it is sometimes difficult to say which type is "normal" and which is "a variant."

individuals which are just viable tend to subdivide into non-viable elements and may, eventually, be eliminated. This artificial method of producing, and finally eliminating, a " sensitive " variant, under the influence of what has been called a " lytic principle," calls for careful consideration, because it may have its counterpart in the animal body, as one of the many and highly complex factors in the mechanism of resistance to bacterial invasion.

As bacteriologists are familiar with the more important work on bacterial variation and transmissible autolysis, there is no need for me to reproduce a detailed review of the literature.

For the immediate purpose of this report a brief historical note will suffice. Opinion on the significance of bacterial variants seems to have been passing through the following phases :—

(1) In ordinary routine work, when an organism is plated out for the purpose of isolating and propagating a pure culture, the bacteriologist selects what he knows from experience to be a typical colony and does not trouble about other colonies which look more or less different from his idea of the normal. The question of variants he regards as negligible.

(2) But patient comparative study of different colonies derived from a single strain, which is known to be pure, has shown that, with most bacterial species, the occurrence of so-called " aberrant " forms is not exceptional but is the general rule and that, therefore, the term " aberrant " may not be justifiable. This is disconcerting, because it means that the forms with which the bacteriologist actually has to deal are not " ear-marked " by nature as " normal " and " variant " but are simply " varieties," each of which may be equally normal. Consequently, one's ideas of what is " typical " and what is a " variant " need justification and may require revision. At this stage it is realised that the question of variants is not negligible; it has to be taken seriously, because much confusion arises from failure to recognise actual, or potential, differences in individuals amongst the bacteria comprised in an ordinary " pure culture."

(3) The study of variants was diverted into a new channel by Twort, who discovered what is now known as " transmissible lytic substance." He first found this substance in growths of micrococci cultured from vaccine virus; it consisted of glassy, transparent material which lysed the micrococci and was transmissible from culture to culture. Subsequently d'Hérelle discovered similar material, which he obtained, in the first instance, from the intestinal excreta of a dysentery patient. These observations have aroused much controversy as to the nature of the lytic substance. D'Hérelle, for example, has always maintained that it is a living, ultra-microscopic virus and calls it a " bacteriophage."

Bordet thinks that it is an autolytic enzyme produced by the bacteria which undergo lysis and that it is due to "a nutritive vitiation primarily started by external influences, an example of which may be the contact with a leucocytic agent." Bail has suggested that it consists of minute particles of the bacteria themselves and that these particles will live and multiply, not independently but when provided with nutrient material from living bacteria. Otto and Winkler regard it not as a living virus but as composed of minute fragments of bacterial protein, arising from the disintegration of living bacteria and endowed with the properties of enzymes. This stage is characterised by controversies between upholders of conflicting theories and, generally, by the assumption that the "Twort-d'Hérelle phenomenon" is an entirely new discovery and differs from previously observed factors concerned with bacterial variation.

(4) Since then a reaction has set in. More attention has been paid to the fact that "lytic substance" is not merely an agent which destroys bacteria; it is also a means of producing bacterial variants. Moreover, it has often been found that some of these variants closely resemble variants described long ago, before "lytic principle" was discovered. Further, it is suggested that "lytic principle" is not a new discovery but simply a revival—supported by new experiments—of old ideas and observations about the lytic properties of bacterial enzymes; and the balance of opinion seems to be definitely against d'Hérelle's view that it is attributable to a living virus which acts as a "bacteriophage."

For my own part, I agree to a large extent with phase (4). In particular, I think that "lytic phenomena" are closely associated with other facts about bacterial variation where lysis may not be demonstrable, and that the former should be considered in conjunction with the latter as part of the same general problem. But, whilst it is desirable to link up new work with old, wherever it is possible to do so, I would not go so far as to suggest that "lytic substance," which acts only on living and actually growing bacteria, may be identified with those previously known bacterial enzymes which are much less restricted in their sphere of action.

In concluding this note, I may give a few references for readers who desire a fuller historical account. Penfold (*Journ. Hyg.*, xi–xiv, 1911–14) has written extensively on bacterial variants and has suggested changes in the enzyme chemistry of the bacterial cell as an explanation of his observations. Arkwright's article (*Journ. Path. & Bact.*, xxiv, p. 36, 1921) is important; it describes his observations on "rough" and "smooth" colonies and brings them into line with other investigators' work on mutation. The question of the "lytic principle" was discussed at the last meeting of the British Medical Association and is

reported, under the title "The Bacteriophage (Bacteriolysin)," in the *British Medical Journal* for August 19th, 1922. More recently (November, 1922), *Medical Science Abstracts and Reviews* has summarised the literature on this subject under the title of "The d'Hérelle Phenomenon."

I think it will be agreed that the whole subject is at present in a state of much uncertainty. There is a multitude of observations on bacterial variants, but no general explanation of the way in which they arise; and it is often impossible to find any correlation between a particular type of variant and a particular kind of disturbing influence. Work on the "lytic principle" is particularly difficult to follow, because there are so many conflicting theories as to the nature of this principle. As the whole subject is very far from being thoroughly worked out, this confusion is only what was to be expected at the present stage.

In the hope that further discussion may help to clear the ground, I put forward the following hypothesis (pp. 17–23) as a possible explanation of some of the phenomena associated with bacterial variation. It seems to me that the essential problem concerns the physiology of the bacterial protoplasm. After developing my hypothesis on this basis, I proceed to illustrate it by recent laboratory data.

Facts to be Correlated.

The main facts to be correlated are the following:—

(1) Bacteria "breed true" to their species; any variations which occur are within the limits of the species. There is practically no evidence which would make it worth one's while to give serious consideration to the possibility of transmutation of species.* It need not be assumed that, when the living bacterial cell divides into two, each portion necessarily retains the species characters; but it does appear to be a tenable hypothesis that any new individual which does not retain characters requisite for identification of its species is not viable, the limits of variation being thus determined by the limits of viability.

(2) The variations which are found are not, as a rule, irregular or haphazard but tend to some one definite direction. This is particularly the case when the change is due to a single influence and not to a variety of factors. The first stage in the process is generally the production of a mixture. The "normal" strain (*a*) produces a mixture of (*a*) forms, which all resemble the original, and of (*b*) forms, which are alike among themselves and possess one or more attributes differentiating them from (*a*). In imagining how this result comes about, there are two possibilities to consider. An (*a*) form may subdivide into an (*a*) and a (*b*);

* I do not propose to enter here into academical questions about the precise definition of "species" as applied to bacteria.

or it may subdivide into two portions, only one of which, either (*a*) or (*b*), is viable. I think this latter possibility is worth bearing in mind. Continuation of the influence which produced the mixture may result in elimination of the one form and survival of a pure culture of the other.

(3) Starting with a pure strain of *b* as the "normal," it may be possible to grow it under conditions which cause it to throw off variants (*c*), all resembling each other and differing in the same respects from *b*. And this may not be the final limit to the possible number of variants. By appropriate treatment of *c*, another variant may be split off, and so on. But a multiplicity of variants does not mean that they are produced in a haphazard sort of way; they seem rather to emerge one at a time, as a series of consecutive reactions between the modified bacterium and its environment.

(4) Under special conditions the characters of the variant recovered from *b* may be those of *a*, *i.e.*, reversion to the original type may occur. Such reversion is most commonly observed when the original influence which converted *a* into *b* was in operation only for a short time.

(5) In the initiation of bacterial variation, a bacterium (A) may divide in such a way that one half (the variant) is not viable, whilst a fellow bacterium (B) may divide into halves which are each non-viable. If all the bacteria behaved like B, the production of variants would mean death of the culture. But suppose that, whilst a large majority of the bacteria behave like B, the remaining few behave like A; the result will be that disappearance of the variant is associated with scanty growth of the more resistant survivors of the normal individuals. This appears to be what happens in the production of "autolysis" associated with the growth of bacteria. It seems to be a special example of bacterial variation, due to an influence which is not an ordinary lysin, because it acts only on bacteria in active growth; it is a selective stimulus, the nature of which will be discussed later, causing the bacteria to subdivide into non-viable elements with the survival of some resistant individuals. For the sake of simplicity, I have stated the case as above; but there is usually an intermediate stage before the variants disappear entirely; some of them are just viable, though tending to subdivide into non-viable forms; if removed from the lytic stimulus at this stage, they may be cultured as a variant characterised by its high sensitiveness to lytic influence.

Correlation of the Facts.

Is there any interrelationship between the data set out above? Perhaps a clue may be found in considering the intimate way in which two essential processes of bacterial life are dependent

on each other, viz., the enzyme action which breaks up the food supply into particles suitable for assimilation and the synthesis of these particles to form living protoplasm.

Considered apart from their association with living matter, enzymes are catalytic agents and their action involves an intermediate stage, during which they form an unstable combination with the material (substrate) undergoing digestion; then follows dissociation of the digested substrate from the enzymes, leaving the latter free to attack fresh substrate. But this is not necessarily the whole story. Conditions may supervene which tend to stabilise (instead of dissociating) the union between enzyme and digested food material, the result being that enzyme action ceases to be progressive and is replaced by a synthesis between substances formerly acting as enzymes and material upon which they acted.

Conditions of this latter nature seem to be of particular importance for cellular growth, in explanation of which it is necessary to postulate a balance of two opposite factors—(1) enzyme action, and (2) inhibition of this action; without the aid of (1) the cell would die from starvation; without the restraining action of (2) it would die from " autolysis." Applying this conception to the enzyme action of bacterial protoplasm, it would appear that bacterial substances which act as catalytic agents are also concerned with the synthesis of bacterial protein, and that bacterial protoplasm may be regarded as a complex of enzymes and the products of enzyme action, a complex which involves the synthesis of these products (*e.g.*, amino-acids) into proteins.

Starting with this idea that the cellular enzymes of bacteria are not merely catalytic agents acting on a substrate, but are also the nucleus upon which bacterial protoplasm is built up, it would seem that, when a bacterium begins to grow, catalytic action upon its environment is at first predominant; then it passes through a critical phase which fixes or stabilises the constituents of bacterial individuality, a phase which is marked by the transition from catalytic action on the raw substrate to synthetic action on the elaborated product. When full development is followed by division, these processes are started afresh.

As bacteria breed true to species, it must be assumed that, in the mechanism of growth and reproduction, there is a constant repetition of the same processes, with identity of catalytic agents, of products ready for assimilation, and of their synthetic combination. This precise uniformity becomes easier to understand with the help of the idea that both catalytic and synthetic action are attributes of the same protoplasmic substances.

Then where is one to find opportunities for variation (within the limits of species characteristics)? Here the incidence of what I have termed the " critical phase " in the transition from catalysis to synthesis may be worth considering, as providing

such opportunities. When a young culture is growing under favourable conditions, without the production of any variants, it may be presumed that the stabilising influence which promotes synthesis acts at exactly the right time and in the right way. But under less favourable circumstances, this influence may begin to operate (*a*) a little too soon, or (*b*) a little too late, or (*c*) much too late; and the consequences may be, respectively (*a*) failure of some of the bacteria to develop their full biological properties, *e.g.*, loss of some function or of some antigenic constituent, (*b*) the acquisition (after full development of biological potentialities) of some additional chemical groups which temporarily mask one or more of these potentialities, *e.g.*, disappearance and subsequent return of some biological property, (*c*) failure to build up the new protoplasm requisite for growth, with " autolysis " as the result. And one might amplify these possible causes of variation by imagining that the stabilising influence did not act " quite in the right way," *i.e.*, that it caused some deviation from the normal molecular or colloidal arrangements for the synthesis of chemical groups into protein.

Is it possible to explain in more definite terms the idea that certain variations in bacterial life are attributable to irregularities in the action of a " stabilising influence " ? I think it is; but one must not be expected to perform impossibilities. No physiologist can explain all the activities of living protoplasm; and bacteriologists are equally unable to give the precise reasons why a bacterium grows or why it grows in a particular way. All that can be done is to note certain influences which probably participate in the phenomena of bacterial life.

I propose first to describe some of these suggested influences in general terms and then to illustrate them by concrete examples.*

It is convenient to consider separately—I. The initial stimulus to variation and II. The propagation of the variant.

I. The causes which may initiate variation are many and diverse. Roughly and provisionally they may be divided into (*a*) those which are obviously non-specific, *i.e.*, where there can be no structural relationship of the " lock and key " type between the stimulus and the bacterial protoplasm, and (*b*) specific influences, where the stimulus has, directly or indirectly, a special selective action, due to structural relationship, upon the bacterium or upon the environment of the bacterium.

Under (*a*) are included a large number of purely physical influences, such as a slight change in temperature, or in the reaction of the medium, or in the colloidal balance between the bacterial constituents and their environment. Exactly how each influence operates cannot be explained, but it is known that the normal arrangement of the particles which are being synthe-

* Perhaps readers not already familiar with the subject will find the argument easier to follow if they take the concrete examples (pp. 23–33) first. This, of course, is not the correct logical sequence, as the examples are not the basis of the more general statement, but merely illustrations of it.

sised by the bacterial protoplasm is often disturbed by purely physical causes.

A good example of (*b*) is the production of a variant by growth in homologous immune serum. Here the conditions are simpler and it is possible to explain the stimulus which initiates the variation as being directly due to the specific antibodies in the serum. In this case it is reasonable to postulate that, when digested food particles are being synthesised into protoplasm, the antibodies find in some of those particles their appropriate antigen and " pick them off "; the new bacterium is synthesised, but it is an impoverished bacterium (a variant), because it has been robbed of some of its antigenic components. To take another example, the products of bacterial growth are sometimes highly specific for the bacterium in question; when these products are removed by filtration, growth of the bacterium in this medium (the filtrate) may initiate the production of a variant. How this comes about is not really known; one can only guess. Perhaps it may be said that particles in the bacterial filtrate have receptors identical with those of the living bacterial protoplasm and therewith " pick up " particles ready for assimilation by bacteria in the nascent stage of growth; such bacteria are impoverished, *i.e.*, they are a variant; they may be so much impoverished that their offspring are non-viable.

I have said that the initial stimulus may be (*a*) non-specific, or (*b*) specific. But these are not the only alternatives. It may be partly the one and partly the other; or it may act like (*b*) on one occasion and like (*a*) on another. To revert to the last example, a bacterial filtrate may be so highly specific that it can only produce a variant when acting on its homologous strain; other filtrates may act on all strains of the same species, others on allied species, others, again, on some species which have no relationship to the bacterium producing the active principle contained in the filtrate.

II. When once a new variant is formed, its propagation is obviously due to a spec fic influence, which is the special attribute of the modified bacterial protoplasm. The new arrangement of particles and side-chains usually tends to repeat itself automatically, from generation to generation and from culture to sub-culture, without the need of any external influence. But in the type of variation which is associated with " transmissible autolysis " an external influence is still in operation. Two processes are at work simultaneously; there is renewal of an external stimulus (now specific) for the initiation of the variant, and there is the tendency for the variant to multiply automatically, though with the production of many non-viable forms. To put it briefly, the starting point is a stimulus, either non-specific or specific, causing the formation of variants, some of which are non-viable. These latter disintegrate into particles which act as specific stimuli (like the bacterial filtrates mentioned above) and change more normal bacteria into variants; many of these are non-viable and

disintegrate, with the liberation of more "lytic substance"; so the process is repeated, the net result being rapid "growth" of "lytic substance." At the same time those of the variants which are viable subdivide into a varying proportion of viable and non-viable forms, from the latter of which the supply of "lytic substance" is again increased.

Experiments with lytic substances raise interesting, and often puzzling, questions as to the interplay between specific and non-specific factors in the production of variants.

Here are some instances. A lytic substance derived from a particular strain may (1) act on all strains of the same species, or (2) it may fail to act on some of the strains. Is (1) due to identity, and (2) to lack of identity in the receptor apparatus? That would seem the simplest explanation. Further, when the lytic substance acts on some other species, as well as on the species from which it was derived, is that due to community of "group" receptors? This may be the case, but it is not so easily acceptable. Here "group" receptors, in the purely chemical sense of the term, may have to give way to more physical conceptions; for example, the colloidal condition of bacterial protoplasm may differ in different bacterial species and may be influenced in some cases, but not in others, by the finely-divided colloids in the filtrate containing a particular lytic principle.

Still, it is worth considering how far it may be possible to apply the "receptor theory." Different stimuli may be equally effective in causing a culture to produce lytic substance. For example, a stimulus, a, may cause a normal culture of bacillus C to produce lytic substance c^1, which may be transmitted to a fresh normal culture, and so on, indefinitely. Another stimulus, b, may produce the same effect on bacillus C, yielding a supply of lytic substance c^2, which may be propagated *ad infinitum*. One might suppose that, after several transmissions through identical cultures of normal bacillus C, c^1 and c^2 would be completely identical with each other. But this may not be the case. If stimulus a was a filtrate from a culture of bacillus A (an organism different from C), whilst stimulus b was derived from some other source, and if the lytic properties of the propagated c^1 and c^2 be tested on a culture of A, it may be found that c^1 produces lysis, and that c^2 fails to do so. How does c^1 "remember" its affinity for A? Did a cause the protoplasm of C to break up in a special way, viz., into particles containing receptors for both C and A? And was this principle perpetuated in the transmission of c^1 from culture to culture of C?

This idea of linking up of two (or more) kinds of receptors leads to a further question. By immunisation with bacterial filtrates containing a lytic principle, "antilytic" sera, *i.e.*, sera which neutralise or inhibit lytic action, may be produced. Some of these neutralisation experiments suggest that one may be dealing with a combination of receptors (A and B), of which one (A) is dominant and the other (B) is in abeyance or "masked."

For example, the lytic action of an active principle containing both A and B may be neutralised by an anti-A serum but not by an anti-B serum. Is this a valid explanation?

Whilst it must be freely admitted that the facts about "transmissible autolysis" are very difficult to understand, it seems to me that the most promising line of explanation lies in a development of the suggestion that these data are merely special instances of a general principle determining bacterial variation.

This view is in harmony with experiments which show that the lytic principle is not merely an agent of destruction; it is also a means of increasing bacterial resistance. In the exercise of this latter property its action is progressive. First it divides the bacteria into some relatively resistant forms (A), which survive, and into more sensitive forms (B), a small proportion of which also survive. The process is repeated with the descendants of A and B; the weaker individuals die out and the stronger survive, perhaps including amongst the latter a few more hardy descendants of what were originally B forms. Carried to its conclusion, the process results in the production of a pure strain of highly resistant A forms, the variant (B) having died out. Thereupon the pure A strain behaves like a normal culture, though more robust than the normal because the tendency to variation has been eliminated; it is no longer lysogenic, because the sensitive variants which, on disintegration, yielded the lytic substance, have disappeared.

At this point it is useful to note that, by using the appropriate laboratory method, one of two opposite results may be achieved in the experimental modification of bacteria. Taking advantage of the fact that a normal culture may be split up into resistant and less resistant forms, it is possible to eliminate either of the two and to produce at will, a pure (or relatively pure) form of either the one or the other.

The practical importance of these laboratory results is that they provide information about some of the factors which determine susceptibility and resistance to bacterial invasion.

They at once raise the question, Do similar influences determine the production of bacterial variants in the animal body?

Significance of the Experimental Data.

Examples of bacterial variation are very numerous and present a wide range of differences. All that can be said about many of them is that they are carefully recorded facts but their significance is obscure and the causes which produce them are unknown. It will be more useful, for the purpose of this report, to select some examples of variants which are clearly of importance in the study of infection and immunity, and to confine one's attention to these.

Spontaneous Variations.

In the first place, valuable information has been obtained from observations on variants which occur "spontaneously,"

i.e., without any effort, on the part of the bacteriologist, to produce them.

Andrewes,* working with the four closely related organisms, *B. paratyphosus* B and C, *B. œrtrycke* and the *Newport* bacillus, found that in ordinary young cultures of undoubted purity there existed side by side two varieties which could be separated by plating out. They differed antigenically in a very pronounced manner. "The one group reacted well with the monospecific "serum, ... while they failed to agglutinate at all with "the allied group serum. The other group behaved in the "reverse manner, agglutinating well with the group serum, "though not to its full titre, while they reacted only weakly "with the monospecific serum." These differences in agglutinability were confirmed by agglutinogenic tests. A serum prepared with a "specific" variety was highly selective for specific strains; whereas a serum prepared with a "non-specific" variety reacted with the homologous strain and with other non-specific strains but "it fails to react with specific strains except the one of its own type, and even here the titre is very low." The differences in agglutinogenic action were the main fact; the slight degree of overlapping was to be explained "on the supposition "that specific strains contain a small amount of group antigen, "and unspecific strains a small amount of specific antigen." It is of particular interest to note that the only differences found were those which were demonstrable by serological tests. "The "phenomenon had nothing to do with the 'rough' and 'smooth' "variants described by Arkwright (1921); all the colonies "were in every other respect alike, and all gave uniformly "turbid broth cultures." Furthermore, Andrewes found that the same phenonenon might occur in the human body. "I had "the opportunity of examining the primary spleen culture "from a woman who had died of Ærtrycke infection; ten colonies "were picked from this plate and equally fell into the two "sharply defined groups." Two other facts are of importance. (1) No intermediate forms occurred; the varieties always fell into one or other of two distinctive groups, the "specific" or the "non-specific." (2) On sub-culture, each type readily changed into the other. "I have at times succeeded in obtaining "two consecutive broth cultures in which the character was "maintained pure, but far more commonly even the second "broth culture shows a mixture of types, and I have only rarely "obtained pure characters on solid media."

The above results are clean-cut and are derived from experiments which were free from complicating factors. They are consistent with the observations of other workers who have shown, by the use of the absorption method, that individual bacteria in a pure culture may differ from each other antigenically, some being less fully equipped than others in the attributes of complete specificity. For example, F. Griffith states in the preceding

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* *Journ. Path. and Bact.*, XXV., p. 505, 1922.

report (p. 11) that, when he was working with meningococci, he plated out a culture and examined 40 colonies which were all identical in appearance, but one differed from the rest in failing to lower the titre of the serum for the parent strain, *i.e.*, it showed "a diminution of antigenic complexity." Schütze,* again, found that he could get "substrains," *i.e.*, strains in which it was shown by absorption that the "antigen mosaic" was "defective," by simply making single cell cultures from a normal strain. In both these examples, as in Andrewes' work, the variants were obtained from young cultures and the only differences between the variant and the normal were those which could be demonstrated by serological tests.

Andrewes' data are a particularly good example of what I consider to be the earliest stage of spontaneous variation. It is found in young cultures and consists of only a slight irregularity in the synthesis of bacterial protoplasm, characterised by one feature only—irregularity in the disappearance and reappearance of a particular antigenic constituent.

I propose now to call attention to a more advanced stage of spontaneous variation. Examples are to be found in the work of Arkwright, Benians and Mary Cowan, to which F. Griffith has made reference above (p. 1). In the present connection the following points are worth noting. The production of the changes noted by Arkwright seems to require a more prolonged influence than is the case with Andrewes' variants; old cultures have been found especially useful for plating out colonies exhibiting the characteristic modifications. And the changes in the variants are more profound and more stable. Their colonies are distinguished by their "rough" or "smooth" appearance. The source of the former, the "R" variants, "has been almost "invariably old broth or agar cultures. They may be obtained "from most strains if a culture which has been kept at room tem- "perature or in the incubator for a month or more is plated out." And, though he found that "R" forms might sometimes be made to revert to "S" by daily subculture, the two types were much less liable to reversion than Andrewes' varieties. Like the latter, the "S" and "R" forms "differ very decidedly in their agglu- "tinating, antigenic and absorbing properties with specific sera," but a new feature is found in association with the "R" form; "it agglutinates in 0·85 per cent. solution of sodium chloride and in broth cultures it forms a deposit leaving the liquid clear." With regard to his "S" and "R" forms of a Shiga strain, Arkwright stated that both "were fatal to rabbits when injected "subcutaneously or intravenously in very small doses, but "no attempt was made to find the M.L.D."

Benians' somewhat earlier observations are worth bringing into line with Arkwright's, as the results are more or less similar. He inoculated a guinea-pig subcutaneously with a mixture of a typical (*a*) Shiga bacillus and mucilage of tragacanth; the

* *Journ. Hyg.*, XX., p. 333, 1921.

animal's local lesion then acted as a culture tube in which the bacillus was allowed to age. On plating after two months, he obtained a mixture of (*a*) colonies and morphologically different (*b*) colonies. When (*b*) was subcultured, it often threw off some (*a*) colonies, "especially soon after it was first isolated." The variant (*b*) "usually sedimented both in broth and saline"; it was inagglutinable and not agglutinogenic, but conferred active immunity against both itself and (*a*) and was quite as pathogenic as (*a*).

The special point of interest in Mary Cowan's subdivision of streptococci into "rough" and "smooth" strains is that the former were much less pathogenic than the latter.

To summarise the above, when the action of the spontaneous modifying influence is prolonged, the following changes make their appearance. The colonies formed by the variants are distinctive; there is more profound alteration in agglutinability and agglutinogenic capacity; there is less tendency of the variants to revert to the parent form, and, finally, there is loss of virulence.

In a still more advanced stage of spontaneous modification there is the production of lytic substance.

Here are some examples. Bail* stated that on three occasions he had obtained lytic substance directly from three old cultures of Flexner's bacillus. Gildemeister† declared that the presence of lytic agent could be demonstrated in a special type of colonies which he had described previously (1917) under the name of "inconstant forms" (Flatterformen). He gave them this name on account of their irregularity and their liablity to change in subculture. He was working with intestinal organisms belonging to the coli, dysentery, typhoid and paratyphoid groups and had isolated his cultures from fæcal material. Otto and Munter‡ also noted that the lytic principle could be obtained from old cultures (aged 3 weeks to 3 months) without the aid of the animal body. Gratia,§ again, has obtained similar results by merely allowing a normal culture of *B. coli* to grow old. "An old agar slant "of *Bacillus coli* shows an uniformly dull film on which appear "very distinctly, here and there, small vitreous colonies." These colonies produced a growth "which possesses a great resistance to the lytic agent," whereas the "dull film" was found to be dead. "At the same time we have had the good fortune to isolate "from a subculture of the original strain a colony of organisms "which are, on the other hand, extremely sensitive to the lytic "agent." The two types grew differently in broth, and the sensitive type was also distinguished from the other by being non-motile and less virulent. Ledingham,‖ after remarking that some strains of bacteria "show lytic changes normally," says :— "I attach great importance to this fact. Dr. Lepper, at the

* *Wien. klin. Wochenschr.*, p. 447. 15th Sept., 1921.
† *Berl. klin. Wochenschr.*, p. 1355. 14th Nov., 1921.
‡ *Deutsch. med. Wochenschr.*, p. 1579. 29th Dec., 1921.
§ *Journ. Exper. Med.*, p. 115, 1921.
‖ *British Med. Journ.*, p. 297. 19th Aug., 1922.

" Lister Institute, recovered from the urine of a case of pyelitis a " coliform strain which behaves in culture as if it were a mixture " of bacilli and ' bacteriophage.' "

I do not think there is any need to quote further instances. If spontaneous production of lytic substance directly by the bacteria had only been observed in rare instances, the true explanation might have been that this substance was not produced *de novo* but was present, though unnoticed, along with the strain used as the starting point of the observations. But this objection will not hold, because these results have been observed repeatedly by different investigators, who have started with pure cultures showing no evidence whatever of the action of a " lytic principle " when growth was allowed to take place under normal conditions.

Experiments with Agents capable of producing Variants.

I take as my starting point the production of pneumococcal variants by growth in immune serum, which is described by F. Griffith in the preceding report. Here the influence which produces the variant can be identified at once; it is the action of the specific " type " antibodies on the growth of the homologous strain. The variant is viable *in vitro* but not *in vivo ;* and its incapacity to thrive in the animal body is shown to be due to loss of those elements in bacterial structure which are associated with capsule formation and production of " specific soluble substance." Hence the action of the serum in protection tests is readily explained; the serum produces a variant which, owing to these defects, is not viable in the animal body. Possibly the therapeutic action which has been demonstrated for Type I antipneumococcal serum is also to be explained, or partially explained, on the same principle.

At the same time it must be recognised that this principle is only applicable under special circumstances and does not go very far as a general explanation of the more important facts about immunity reactions and the production of variants. For example, it is impossible to maintain that recovery from pneumonia, without serum treatment, is due to manufacture, by the patient, of those antibodies which are demonstrable in artificially prepared immune sera. And similar difficulties arise in attempting to show that resistance to infections with other species of parasitic bacteria is due to demonstrable antibodies.

Hence, if the results cannot be explained as due to known and demonstrable antibodies, one is led to consider whether there may not be some other specific factor which operates in the living body and converts the invading organism into a non-viable variant. Antibodies, it should be remembered, are not the only influences which are specific for bacteria. Bacterial attributes, though they may be antigenic (*i.e.*, capable, as foreign protein, of giving rise to antibodies) may also act selectively, and even

specifically, in other ways. They may produce non-viable variants by direct action upon bacterial growth, not by the indirect method of stimulating the animal body to produce antibodies which interfere with that growth. Experimentally there are many ways of producing such variants without the employment of antibodies. One of these methods, the use of " lytic substance," is of particular interest, because what is seen to take place in the test-tube may have some counterpart in the reactions going on in the animal body.

The first fact of importance about " lytic substance " is that, as already mentioned, it may be derived from the bacteria *ab initio*. Further, it is not necessary to wait for the chance of its spontaneous emergence in an old culture. Various experimental methods may be employed. For example, Otto and Winkler* stated that lysin formation could be promoted in many different ways, by adding to the culture filtrates from old cultures, by the treatment of the bacteria with immune serum, and by physical or chemical means (shaking in distilled water or the addition of a small quantity of sublimate). But filtration through a bacterial filter was especially useful and was preferable to centrifugalisation because fewer passages were necessary in order to obtain the lysin. Their method was to heat a broth culture at 58° C., filter, add a few drops of filtrate to new broth containing fresh, living culture, and incubate for 24 hours at 37° C. These steps constituted the first " passage." The process was repeated until the lysin was obtained. Weinberg and Aznar† obtained similar results by the filtration method with B. Shiga. They termed their products " autobacteriolysins " because they were obtained directly and primarily from the bacteria. They also showed, by the method of autolysis in distilled water, that these lysins could be obtained from young cultures as well as from old. They took two loopfuls of a 24 hours' growth, emulsified it in 20 c.c. of sterile distilled water, incubated at 37° C., and then demonstrated the presence of lysin in the filtrate.

Lytic substances are of the same general characters, whether produced directly with culture material or by more complicated methods involving animal inoculation or the use of animal products. They are of bacterial origin but must be regarded as a modification of bacterial constituents since they may not be demonstrable as components of normal bacterial protoplasm. This last fact may be illustrated by serological methods.

For example, Bordet and Ciuca‡ thoroughly immunised rabbits with a normal strain of *B. coli* and found that the serum had no antilytic properties. From the same strain, however, they obtained a lytic principle and produced a lysogenic variant. The serum of rabbits immunised with this variant was antilytic. Their inference was that lytic power was undoubtedly a newly acquired character.

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* *Deutsch. med. Wochenschr.*, p. 383. 24th March, 1922.

† *C.R. Soc., Biol.* LXXXVI. (p. 833), 29th Apr., 1922, and LXXXVII. (p. 136), 17th June, 1922.

‡ *C.R. Soc. Biol.*, LXXXIV., p. 280, 29th Jan., 1921.

In the next place it has been shown that " lytic substances " produce variants which are similar, in many important respects, to those occurring naturally or spontaneously in cultures and sometimes in the animal body. It is known, for example, that the effect of the lytic agent upon bacterial growth is to cause a culture to split up into two types, the " sensitive," which is highly susceptible to the action of this agent and rapidly undergoes autolysis under its influence, and the " resistant," *i.e.*, a type which resists this action. Here reference may be made to Gratia's work* on the production of variants with the aid of lytic substances. Starting with a single strain of *B. coli*, he obtained eleven different forms, each with distinctive characteristics though all still retained the specific properties of *B. coli*. In the course of this work, he says, " our attention has been called repeatedly to similar facts reported by Arkwright." He refers to Arkwright's observations on " rough " and " smooth " variants which are discussed above by F. Griffith and me (p. 5 and p. 25).

Significance is to be attached to the fact that the initial stimulus which produces transmissible " lytic substance " may be derived from many quite different kinds of material, *e.g.*, fæcal extracts from healthy or diseased persons or from healthy animals of various species, urine, diluted sewage, extracts of normal organs, peritoneal exudates of guinea-pigs inoculated with some bacterium, mixtures of bacterial culture and leucocytes, and so on. It is of much interest to find that, whether the modification arises " spontaneously " or under the influence of one or other of these heterogeneous " lytic influences," the kinds of variants which are produced are always very much the same. The reason, I think, is that the modifying influence, whatever its nature, always acts in the same sort of way, viz., by interfering with the normal synthetic activities of bacterial protoplasm at the critical time when the growing bacterium is about to subdivide.

Something may now be said about the transmissible properties of lytic substance, which, since they are of bacterial origin, will naturally depend on the characters of the bacterial protoplasm from which they were derived.

Sometimes the individuality of the strain from which they originated is marked very strongly. A good example is given by Gratia.† He started with a lytic substance (bacterial filtrate) which acted only on the particular strain of *B. coli* from which it was derived. It was " without any action not only on other closely " related species but also on other strains of *Bacillus coli*."

He found, however, that he could make it produce a lytic substance possessing a wider range of action. From his original strain of *B. coli* he separated out two types, the " sensitive " and the relatively " resistant," upon both of which his first lytic filtrate produced lysis. With the filtrate from the lysed " resistant " strain, " a very marked dissolution was observed of

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* *Journ. Exper. Med.*, XXXV., p. 287, 1922.

† *Journ. Exper. Med.*, XXIV., p. 115, 1921.

" Shiga bacilli, Flexner bacilli, Hiss Y type of Flexner bacilli, and " also a strain of *B. coli communis* which was unattacked by the " original filtrate." He further remarks that, in consequence of these observations, which were made by Wollstein, " we have " been able, by a method of successive passages through appro- " priate strains, to extend the lytic power to other species, as " typhoid and paratyphoid bacilli, and have obtained by this " somewhat different technique results similar to those recently " published by Bordet and Ciuca."

I think the point of special importance in the above observations is that the transition from the lytic principle (*a*), which acts only on its own strain, to the wider lytic influence (*b*) is effected without the introduction of any new factor. It is simply the result of the continued action of *a*, which first produces relatively resistant forms of the original strain and then, by attacking and breaking down some of these resistant forms, gives rise to the wider lytic principle *b*. This means, expressed in terms of the hypothesis which I am advocating, that the synthesis of the bacterial protoplasm is interrupted more readily, or at an earlier stage, in the case of the normal bacillus than in the case of the resistant form; with the latter, synthesis has gone a little further before the lytic principle succeeds in producing its disintegrating effect, and, consequently, the products of disintegration are different.

From this point of view one may attempt to throw a little light on the puzzling question, Why are there such remarkable differences amongst what I may term " pure " lytic principles, which are derived from different strains of the same bacterial species? By " pure " I mean that each is derived *ab initio* from a particular strain of the species and has not been subjected to any external modifying influence, such as passage through another strain of the species or through a strain of another species. I think the explanation, at least in part, is to be referred to the extreme complexity of protoplasm, owing to which very slight differences in its stability, or in the particular arrangement of its constituent parts, at the critical phase of bacterial growth, may result in disintegration products possessing different lytic activities.

I think it is desirable first to recognise such possible complexities of a single species, before embarking on still more complicated problems such as (1) Why is a " coli " lytic principle altered when it is transmitted through a culture of a different species, such as Shiga, or (2) Does transmission of lytic principles through different representatives of intestinal bacteria bring out evidence of " group " relationship and different degrees of kinship?

Here is another interesting study which throws some light on " lytic principles," because the experimental conditions are not too complicated. It is concerned with the end results which are obtained by continued exposure of bacteria to a lytic agent.

Martha Wollstein,* starting with the fact that the appropriate "bacteriophage" for dysentery bacilli causes these organisms, when plated out, to divide up into "regular" and "irregular" colonies, compared the action of the lytic principle on cultures of each of these types. It was found that the "regular" strains were more resistant than the "irregular." Tests with the filtrates from these strains showed that the lytic principle was carried by the latter but not by the former. Both the regular and the irregular colonies were subcultured on plates for more than 40 generations. The former retained their characters without any alteration; the latter approximated to the former type, until more regular than irregular colonies were present. "The explanation seems to be that the sensitive bacilli die off more rapidly than the resistant ones, which form the regular colonies in later generations. It is a matter of selection." She then endeavoured to ascertain whether the acquired lysogenic property was retained permanently. "Working with the original, normal culture as a whole, it was found that after the seventh generation on agar or in broth the bacilli which had survived contact with a lytic fluid were no longer able to transmit the lysogenic property to other cultures or to dissolve the normal Shiga bacilli." As regards the two main properties of the regular colonies, resistance to lysis and loss of lysogenic power, it was found in some experiments that a certain amount of lysis was produced on bacilli cultured from regular colonies. The explanation was that these cultures were not absolutely "pure," *i.e.*, some less resistant bacilli were still present. This was shown by re-plating, when both regular and irregular colonies developed from a broth culture of the strain in question, which had been in contact with lytic fluid over night. The regular colonies picked from this plate were completely resistant to lytic action. Put briefly, the results of the action of a lytic principle were :—(1) production of strains which resisted lytic action, were not lysogenic, and were not agglutinable; (2) production of strains which were lysogenic, agglutinable, and sensitive to lytic action; (3) gradual conversion of these latter strains into the resistant and non-lysogenic type.

If a lytic principle (*a*) derived from bacillus A is found to act on a different bacillus B, it is not surprising that the lytic principle (*b*) derived from filtrates of B may differ from *a* in its range of action. But it is a curious fact (mentioned on p. 22) that *b*, even after many transmissions through cultures of B, may retain a certain impress of the individuality derived from A. This individuality may be demonstrated by immunising animals with a lytic substance and obtaining in their serum the corresponding specific antibody. Two examples are worth quoting. Bruynoghe and Appelmans† prepared antisera with two typhoid "bacteriophages," the one obtained from Strasburg and the other from Louvain. The latter was originally a coli "bacteriophage" but

* *Journ. Exper. Med.*, XXXIV., p. 467, 1921.

† *C.R. Soc. Biol.*, LXXXVII., p. 96, 27th May, 1921.

had been adapted to the typhoid bacillus and had lost all action on coli. The antiserum obtained from the latter neutralised not only the Louvain " bacteriophage " but also its predecessor, the coli " bacteriophage "; it had no action on the Strasburg " bacteriophage." The Strasburg antiserum neutralised only the Strasburg " bacteriophage." Gratia and Namur* obtained somewhat similar results with antisera for substances which were lytic for staphylococci. The one substance (BH) was lytic primarily for *Staphylococcus aureus* (H) and the other (BV) for *Staphylococcus albus* (V); but, whereas BH acted on many strains of these organisms, including V, BV was strictly specific for V. The antiserum prepared with BH was specific for BH alone and the antiserum for BV was equally selective for BV. After the lytic principle BH had been allowed to act on V and had been transmitted ten times through V, the action of the two antisera was tested on the lytic substance finally obtained. It was found that no change had taken place; specific neutralisation was still obtained by the BH antiserum but not by the BV antiserum.

How do these antilytic sera act? Do they behave like ordinary antibodies which combine with specific constituents corresponding to those of the antigen used for immunisation? Apparently they do; at least, there is no reason to suppose that they are an exception to the ordinary rule. In this connection the following absorption experiments are worth noting. Jaumain and Meuleman† placed in each of three tubes 5 c.c. of a lytic principle active for staphylococci and added to tube (*a*) 10 drops of broth, to (*b*) a like quantity of coli culture killed by heat, and to (*c*) the same amount of staphylococci killed in the same way. The tubes were sealed and kept in the incubator for three days. Then the contents were tested quantitatively for lytic action on staphylococci; (*a*) and (*b*) had lost none of their activity, but (*c*) was 1,000 times weaker. A few days later, filtrates from the three tubes were tested; (*a*) and (*b*) were as potent as the original; (*c*) was inactive. They stated that similar results, *viz.*, absorption of active principle by killed homologous bacteria, had been obtained by Costa Cruz with *B. coli*. This absorption, they found, was strictly specific and was not obtained if the killed bacteria, though belonging to the same species, were derived from a strain not sensitive to the lytic principle.

I mentioned above (p. 21) that it is convenient to draw a distinction between the initial stimulus, which starts the production of transmissible lytic material, and this material itself. The former may be some non-specific chemical or physical influence; the latter is definitely a bacterial product. This distinction is worth bearing in mind when one comes to consider the significance of the lytic material which is obtained not from the test-tube but from the animal body.

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* *C.R. Soc. Biol.*, LXXXVII., p. 364. 24th June, 1922.
† *C.R. Soc. Biol.*, LXXXVII., p. 362. 24th June, 1922.

Several observers have found that lytic material is present in the serum of an animal shortly after inoculation with a small dose of bacteria. To take a recently published example, Otto, Munter, and Winkler* inoculated a guinea-pig intraperitoneally with one-twentieth of a loopful of Flexner bacilli and found that lytic principle was present in the serum obtained by heart puncture seven hours afterwards. No lytic material was present in the control (serum of the same guinea-pig obtained shortly before inoculation with bacilli). How is the activity of the later sample of serum to be explained? Is it due to the presence of bacterial products, derived from the Flexner bacilli? Or does it mean that the bacteria produced in the animal's plasma a temporary chemico-physical change (Allergie), and that this changed condition, being present in the serum, was operative *in vitro* as a primary stimulus causing a growing culture to produce lytic substance?

Similar phenomena have often been observed after injecting animals with lysogenic bacterial extracts. Bordet and Ciuca,† for example, injected a guinea-pig subcutaneously with 2 c.c. of "lytic liquid" (a lysed and filtered suspension of *B. coli*) and bled the animal seven hours afterwards. Twelve drops of the serum, heated for half an hour at 56° C., prevented the growth of a culture of *B. coli*, whereas the control serum, taken before inoculation was ineffective. Similar results were obtained with the serum of a rabbit taken from 7 to 24 hours after intravenous injection with 20 c.c. of "lytic liquid." Here, again, the question is whether the serum simply contained some of the original lytic substance, which was still active, though highly diluted, or whether the bacterial extract had modified the serum in a way similar to that suggested at the end of the preceding paragraph.

In the same article Bordet and Ciuca raise a similar problem about the transmission of antibodies. It is worth quoting, though not strictly apposite to the present question (transmission of lytic substance). After withdrawing a little blood from a guinea-pig weighing 600 grammes, they inoculated the animal subcutaneously with 5 c.c. of an antilytic serum and bled it 32 hours subsequently. The two sera thus obtained were heated at 56° C., and then two drops of each were tested for antilytic power; the former serum was inactive, the latter active.

I am not prepared to offer any cut-and-dried explanation of these phenomena. The main fact appears to be that bacterial variants, associated with lytic change, are produced in the animal body as well as in the test-tube. Whether the initial stimulus which produced them is bacterial or animal, their propagation depends on the bacteria themselves, *i.e.*, on bacterial growth and on the action of bacterial products. This is the point which I wish to emphasise for the purpose of the subject now under discussion. It would be beyond the scope of the present report to enter into important collateral questions about the part played by the animal body in resistance to bacterial infection.

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* *Zeitschr. f. Hyg.*, XCVI., p. 118, 1922.

† *C.R. Soc. Biol.*, LXXXIV., p. 280. 29th Jan., 1922.

Summary.

It is now recognised that the study of bacterial variation is of practical importance, because the subject is concerned with variations in the capacity of bacteria for producing disease.

One is confronted with a confusing mass of data, ranging from some minor and temporary change in a single function to the much more profound alterations, popularly known as "the Twort-d'Hérelle phenomenon," where the vitality of the bacteria is seriously imperilled and relatively few individuals survive.

It would simplify the matter if one could recognise some general principle underlying this apparent confusion, and could attribute the different types of variants to different phases in the operation of this principle.

In the search for a common factor, it is observed that these changes only occur with living and actually growing bacteria and that, therefore, they are probably due to some influence which operates at the nascent stage of growth. Hence the common factor can only be explained in terms of vital processes; but about these no precise information is available.

Still, it is possible to formulate a general statement. The constituents of living bacterial protoplasm have two functions, catalytic action, *i.e.*, the preparation of food material with the aid of their appropriate enzymes, and synthetic action, *i.e.*, the building up of new protoplasm. It is obvious that the balance between these two activities must be very minutely adjusted and that any disturbance of this balance will tend to the production of variants.

My report is concerned with the development of this theme in relation to known facts about bacterial variants.

January, 1923.

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